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Photosynthetic Response to Elevated CO_2 in Poplar (POP-EUROFACE) in Relation to Leaf Nitrogen Partitioning

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Abstract

We examined the effect of elevated CO_2 on photosynthesis and the allocation of leaf nitrogen to photosynthetic and non-photosynthetic pools in two poplar species (*Populus alba,* genotype 2AS11 and *P. nigra,* genotype Jean Pourtet) at the POP-EUROFACE site. In *P. alba,* the light-saturated photosynthetic rate at growth CO_2 concentration (A_{sat}) over two years was significantly higher (by 42%) in trees exposed to CO_2 enrichment. In *P. nigra,* there was no significant difference, though on average A_{sat} was 29% higher in the CO_2 enrichment treatment. Stomatal conductance was significantly reduced by CO_2 enrichment in both species: by 22% in *P. alba* and by 18% in *P. nigra.* Neither maximum carboxylation rate (V_{cmax}) nor maximum rate of electron transport ($J_{max)}$ was reduced by CO_2 enrichment. A change in the partitioning of leaf nitrogen between photosynthetic and non-photosynthetic pools was detected in *P. nigra*: partitioning into non-photosynthetic nitrogen increased by 15% in elevated CO_2 conditions. No differences were detected between the sun and shade leaves of either studied species in terms of their photosynthetic responses to elevated CO_2. The greater allocation of leaf nitrogen into the non-photosynthetic pool in elevated CO_2 conditions displayed by *P. nigra* in comparison with *P. alba* may explain the non-significant photosynthetic stimulation in the former.

Key words: net CO_2 assimilation, nitrogen partitioning, non-photosynthetic nitrogen, photosynthetic down-regulation, *Populus*, stomatal conductance

Introduction

Plants generally respond to rising CO_2 concentration via reduced stomatal conductance and increased photosynthesis. The magnitude and persistence of photosynthetic stimulation is important in determining the resulting increment in biomass accumulation. However, photosynthetic down-regulation (acclimation) has also been reported in many experiments (Medlyn et al. 1999, Norby et al. 1999): plants grown in high CO_2 may exhibit a lower photosynthetic rate compared with plants grown in ambient CO_2 when both groups are measured at a common CO_2 concentration. Down-regulation in leaves grown in elevated CO_2 may be associated with adjustments at the biochemical level, which is evidence of a reduction in maximum carboxylation rate (V_{cmax}; see Table 1 for abbreviations) or maximum rate of electron transport (J_{max}) (Medlyn et al. 1999, Ellsworth et al. 2004). Reduced leaf nitrogen (N) concentration and a downward shift in the relationships between photosynthetic capacity (V_{cmax} and J_{max}) and leaf N may also be involved in biochemical down-regulation in elevated CO_2 (Medlyn et al. 1999, Ellsworth et al. 2004). In addition, sink limitation and accumulation of carbohydrates have been associated with photosynthetic acclimation (Drake et al. 1997, Moore et al. 1999). Thus, as concluded by Medlyn et al. (1999), multiple factors appear to be involved in the down-regulation of photosynthesis in elevated CO_2 conditions.

Not all leaf N is directly involved in photosynthesis; it is also present in non-photosynthetic pro-

Table 1. List of symbols and abbreviations

Symbol	Definition
A_{sat}	Light-saturated net CO_2 assimilation at growth c_a (μmol m^{-2} s^{-1})
A_{400}	Light-saturated net CO_2 assimilation at c_a of 400 μmol mol^{-1} (μmol m^{-2} s^{-1})
c_a	Ambient CO_2 concentration (μmol mol^{-1})
c_i	Leaf intercellular CO_2 concentration (μmol mol^{-1})
D_s	number of stomata per unit leaf area (mm^{-2})
FACE	Free-air CO_2 enrichment
g_s	Stomatal conductance (mmol m^{-2} s^{-1})
ISF	Indirect site factor
J_{max}	Maximum rate of electron transport (μmol electrons m^{-2} s^{-1})
LMA	Leaf mass per area (g m^{-2})
N_A	Leaf area-based nitrogen content (g m^{-2})
N_M	Leaf mass-based nitrogen concentration (%)
PPFD	Photosynthetic photon flux density (μmol photons m^{-2} s^{-1})
pl	stomatal pore length (μm)
P_B	Partitioning of leaf N into bioenergetics associated with electron transport
P_L	Partitioning of leaf N into light harvesting
P_{non}	Partitioning of leaf N into non-photosynthetic nitrogen pools
P_R	Partitioning of leaf N into carboxylation
SPAD	Index of relative chlorophyll content
V_{cmax}	Maximum carboxylation rate (μmol CO_2 m^{-2} s^{-1})

teins, leaf structural components, nucleic and amino acids and secondary compounds. This non-photosynthetic N fraction accounts for 50-60% of total leaf N (Evans 1989, Onoda et al. 2004, Takashima et al. 2004, Eichelmann et al. 2005). If the proportion of non-photosynthetic N increases in elevated CO_2 concentrations, as has been shown for *Eucalyptus cladocalyx* (Gleadow et al. 1998), photosynthesis may be biochemically down-regulated even if there is no reduction in total leaf N. Niinemets and Tenhunen (1997) proposed a model to calculate the partitioning of leaf N into different photosynthetic functions. Using this model, the non-photosynthetic N portion (P_{non}) is the residual of the photosynthetic N budget.

Recent reviews have concluded that long-term photosynthetic enhancement in elevated CO_2 conditions is greater in woody than in herbaceous species (Nowak et al. 2004, Ainsworth and Long 2005, Ainsworth and Rogers 2007). The poplar free-air CO_2 enrichment experiment (POP-EUROFACE) was initiated in Italy in 1999 to investigate the effect of elevated CO_2 on *Populus alba, P. x euramericana* and *P. nigra* (Miglietta et al. 2001). Poplars are productive species with indeterminate growth patterns and high sink strength, and are used for biomass production in short rotation forestry. During the second rotation, trees planted at the EUROFACE site produced significantly more above-ground biomass at elevated compared with ambient CO_2 levels (< 31 Mg ha^{-1} y^{-1} and < 26 Mg ha^{-1} y^{-1}, respectively; Liberloo et al. 2006). Published EUROFACE results concerning the magnitude and persistence of photosynthetic responses have generally reported high and sustained stimulation (Liberloo et al. 2007) with only occasional down-regulation detected during re-growth after the first coppice (Bernacchi et al. 2003) or in shade leaves at the end of the growing season (Calfapietra et al. 2005).

The present study aimed to examine the vertical profile of photosynthetic responses to elevated CO_2. Such information could assist attempts to model the responses of foliage in vertical profile of the canopy to elevated CO_2. The following specific questions were asked: 1) Is photosynthetic stimulation in elevated CO_2 sustained in all canopy layers with no evidence of down-regulation? 2) Does elevated CO_2 affect the partitioning of leaf N between photosynthetic and non-photosynthetic pools? 3) Does a correlation exist between photosynthetic response and changes in leaf N partitioning?

Materials and Methods

Experimental site

The experimental site, located in Tuscania, Central Italy (N 42°22', E 11°48'), contained six circular plots (diameter 22 m): three plots for an elevated CO_2 treatment (daytime target CO_2 concentration of 550 μmol mol^{-1}) and three for an ambient CO_2 treatment (CO_2 concentration 380 μmol mol^{-1}). Each plot was divided into six equal sectors and planted with three poplar species (planting density 10,000 trees ha^{-1}), two sectors per species: *P. alba* L. (genotype 2AS11), *P. nigra* L. (genotype Jean Pourtet) and *P. x euramericana* (Dode) Guinier (genotype I-214). Details of the FACE design have been described by Miglietta et al. (2001), and the plantation layout and the clonal properties have been characterized by Calfapietra et al. (2001). The study was performed during the second and third growth years of the second rotation: 2003 and 2004. During the second rotation, additional fertilization was added to three sectors of each experimental plot through the drip irrigation system. The total amount of N supplied was 212-290 kg ha^{-1} y^{-1}. High N fertilization was used to counterbalance the large amounts of N removed from the soil after the first coppice (Calfapietra et al. 2007). Only the results for *P. alba* and *P. nigra* are presented in this paper.

Gas exchange measurements

Gas exchange measurements were made between September 5th – 13th, 2003 and September 8th – 22nd, 2004 using CIRAS-2 portable equipment (PP Systems, Hitchin, UK) with a halogen light source. In 2003, photosynthesis measurements were performed on the leaves from the uppermost quarter of the crown (hereafter referred to as sun leaves) and from the lowest quarter (shade leaves). In 2004, a middle canopy layer was added. Average ISF (indirect site factor) values were over 0.50 and below 0.25 for the uppermost and lower layers, respectively, being between 0.25 and 0.50 for the middle layer. For each species × CO_2 level × fertilization level × canopy layer combination, three leaves were cut at comparable positions along the branches under degassed water and inserted into the gas exchange system. Leaf temperature was held at 25°C and photosynthetic photon flux density (PPFD) was maintained at 1,000 mol m^{-2} s^{-1} during the measurement of A/c_i curves (net CO_2 assimilation rate, A, versus internal CO_2 concentration, c_i). PPFD of 1000 mol m^{-2} s^{-1} was chosen to maintain a standard procedure throughout the experiment while avoiding photodamage of shade leaves. Measurements of A were performed starting at growth CO_2 concentration, decreasing stepwise to 50 μmol mol^{-1} and then increasing stepwise to 1200 μmol mol^{-1}. Photosynthetic rates reached a steady state within 2-3 min following a change in ambient CO_2 concentration (c_a). Light-saturated photosynthetic rate (A_{sat}) was taken as the value of photosynthesis at growth CO_2 concentration and PPFD=1000 μmol m^{-2} s^{-1} (i.e. the first datapoint of the A/c_i curve). In order to detect photosynthetic down-regulation, photosynthesis at ambient CO_2 concentration was calculated for the elevated CO_2 treatment as the value of the net assimilation rate at a CO_2 concentration of 400 μmol mol^{-1} in the leaf cuvette (A_{400}). The light-saturated carboxylation rate (V_{cmax}) and the maximum rate of electron transport (J_{max}) were calculated according to von Caemmerer and Farquhar (1981). The values of parameters K_c, K_o (Michaelis-Menten coefficients of Rubisco activity for CO_2 and O_2, respectively) and Γ* (CO_2 compensation point in the absence of mitochondrial respiration) at 25 °C were used to calculate V_{cmax} and J_{max} as in McMurtrie and Wang (1993).

In situ stomatal conductance (g_s) was measured with an AP-4 porometer (Delta-T Devices, UK). The leaves (n=6) selected from two (in 2003) or three (in 2004) canopy layers were measured several times between 10 AM and 5 PM. In 2003, only abaxial leaf surfaces were measured for stomatal conductance, but in 2004, both leaf surfaces were measured, and total stomatal conductance was calculated as the sum of adaxial and abaxial conductances. In *P. alba*, stomata are only present on the abaxial leaf side, so the abaxial data represented total gs in both 2003 and 2004. *P. nigra*, however, is amphistomatous. In 2003, an extra set of measurements on 28 leaves revealed that the ratio of adaxial to abaxial g_s for *P. nigra* was 0.46 (±0.033). The value of total leaf conductance for *P. nigra* was thus calculated as 1.46 times abaxial conductance in 2003.

In 2004, some leaves used for in situ gs measurements were collected to study stomatal morphology (4 leaves from every species × CO_2 level × fertilization level × canopy layer combination). Leaves were coated with clear nail varnish and an imprint was made by pressing adhesive clear tape onto the varnish. Images of the impressions were captured at 400 magnification using a Nikon E50i microscope and a Nikon DS-Fi1 camera (Nikon Instruments Inc., Japan). All morphological measurements were taken half-way from the leaf tip to the base and half-way from the mid-rib to the margin. The number of stomata per unit leaf area (Ds) and stomatal pore length (pl) were determined within three microscope fields of view (0.097 mm^2) using ImageJ software version 1.36b (National Institutes of Health, USA).

Light conditions for each sampled leaf were determined from photographs taken with a Nikon CoolPix 950 digital camera equipped with hemispheric lens. The ratio of diffuse light at leaf level to that above the canopy (ISF) was measured with WinSCANOPY software (Regent Instruments Inc., Canada), assuming a uniform overcast sky.

Analysis of leaf N and chlorophyll

In 2003, chlorophyll concentrations (SPAD values) were measured in leaves subjected to photosynthesis analysis using a Minolta SPAD-502 chlorophyll meter. Leaf mass per area (LMA) and nitrogen per unit leaf mass and area (N_M, % and N_A, g m^{-2}, respectively) were also determined in these leaves. LMA was calculated as the ratio of leaf dry mass (weight of leaves dried at 70°C for 48 hours) to leaf area. Leaf area was determined using a Li-Cor 3100A leaf area meter (Li-Cor Inc., NE, USA). NM was measured using a PE 2400 Series II element analyser (Perkin Elmer, USA). SPAD values were calibrated against a wet-extraction of chlorophyll from a sub-sample of leaves used for stomatal measurements. Wet-extraction of chlorophyll has been described by Calfapietra et al. (2005). Chlorophyll concentration in all leaves used for photosynthetic measurements was then calculated from SPAD values using species- and treatment-specific linear regressions between SPAD and chlorophyll concentration. In 2004, circular disks of 10 mm were cut from the leaves used for photosynthesis measurements in order to wet-extract the chlorophyll (as described by Calfapietra et

al. 2005), while the remainder of the leaves was used to determine LMA and NM as described above. Chlorophyll concentrations were used to calculate P_L (see below).

Partitioning of leaf N into carboxylation (P_R), bioenergetics associated with electron transport (P_B) and thylakoid light-harvesting (P_L) components was calculated according to Niinemets and Tenhunen (1997) using values of V_{cmax}, J_{max} and leaf chlorophyll concentration. The fraction of non-photosynthetic N in leaves (P_{non}) was calculated as:

$$P_{non}=1-P_R-P_B-P_L \quad (1)$$

Statistical analysis

Analysis of variance (GLM procedure) was used to assess the effects of CO_2 level, fertilization level and canopy layer on the studied leaf characteristics. Since the effect of N fertilization on leaf characteristics was always non-significant (owing to high background nutrient availability in the study site, Liberloo et al. 2009), the two N levels were pooled, resulting in six measurements for every species × CO_2 × canopy layer combination. Severe chlorosis was observed in plots 5 (elevated CO_2) and 6 (ambient CO_2) in September 2004 and therefore the data from these plots were excluded from further calculations. The effects were considered significant at $P<0.05$. All statistical analyses were performed with Statistica, version 7.0 (StatSoft Inc., Tulsa, OK, USA).

Results

September 2003

Light availability (ISF) was not significantly influenced by elevated CO_2, indicating that corresponding leaves in ambient and elevated CO_2 treatments were compared under similar light conditions (Table 2). NM was significantly lower in the *P. alba* sun leaves and the *P. nigra* shade leaves grown in elevated CO_2 conditions compared with the corresponding leaves from the ambient CO_2 treatment (Fig. 1, Table 2). The significant interaction between CO_2 treatment and canopy layer for NM and NA in *P. nigra* resulted from opposite responses to elevated CO_2 in sun and shade leaves. A significant positive effect of elevated CO_2 on Asat was only detected in *P. alba* (Fig. 2, Table 2). The effects of CO_2 enrichment on LMA, N_A, A400, V_{cmax} and J_{max} were non-significant in both species (Table 2). Canopy layer strongly influenced LMA, N_M, N_A, A_{sat}, A_{400}, V_{cmax}, J_{max} and g_s, with higher values detected in sun than in corresponding shade leaves (Table 2, Figs 1-2). Expressing photosynthetic characteristics (A_{sat}, V_{cmax}, J_{max}) as a function of leaf mass

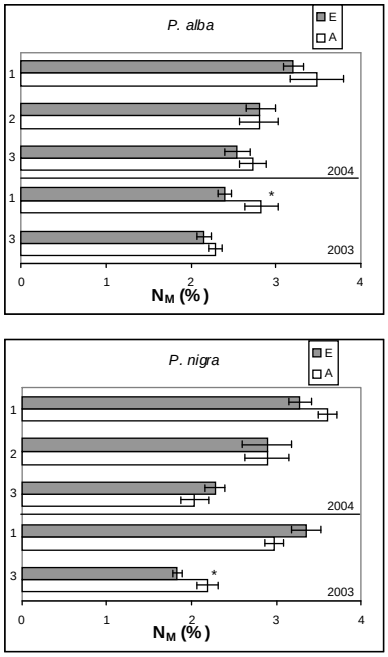

Figure 1. Leaf mass-based nitrogen concentration, NM, (%; average and SE) in leaves from different treatments and canopy layers. Numbers 1-3 on the vertical axis refer to vertical canopy layers: 1=uppermost; 2=middle (in 2004 only); 3=lowest. CO_2 treatments: E=elevated CO_2; A=ambient CO_2. Star (*) refers to a significant difference between corresponding ambient and elevated CO_2 treatment

Table 2. ANOVA results of the effects of CO_2 concentration and canopy layer on leaf characteristics in 2003-2004. ns=not significant. All effects were considered significant at $P<0.05$

		Populus alba			*Populus nigra*		
		CO_2	Canopy layer	CO_2 *layer	CO_2	Canopy layer	CO_2 *layer
N_M	2003	0.033	0.004	ns	ns	0.000	0.009
	2004	ns	0.006	ns	ns	0.000	ns
N_A	2003	ns	0.000	ns	ns	0.000	0.028
	2004	ns	0.000	ns	ns	0.000	ns
LMA	2003	ns	0.000	ns	ns	0.000	ns
	2004	ns	0.000	ns	ns	0.001	ns
A_{sat}	2003	0.036	0.001	ns	ns	0.000	ns
	2004	0.006	0.000	ns	ns	0.001	ns
A_{400}	2003	ns	0.000	ns	ns	0.001	ns
	2004	ns	0.000	ns	ns	0.001	ns
V_{cmax}	2003	ns	0.000	ns	ns	0.000	ns
	2004	ns	0.000	ns	ns	0.000	ns
J_{max}	2003	ns	0.000	ns	ns	0.000	ns
	2004	ns	0.001	ns	ns	0.000	ns
ISF	2003	ns	0.000	0.036	ns	0.000	ns
	2004	ns	0.000	ns	ns	0.000	ns
g_s	2003	0.021	0.000	ns	0.008	0.000	0.042
	2004	0.049	0.000	ns	ns	0.000	ns
D_s	2004	ns	0.000	ns	ns	0.000	ns
pl	2004	ns	0.006	0.010	ns	0.000	ns
P_R	2003	ns	0.027	ns	ns	ns	ns
	2004	ns	ns	0.031	0.016	ns	ns
P_B	2003	ns	ns	ns	ns	ns	ns
	2004	ns	ns	0.016	ns	ns	ns
P_L	2003	ns	0.000	ns	ns	0.000	ns
	2004	ns	0.002	ns	ns	0.000	ns
P_{non}	2003	ns	ns	ns	ns	ns	ns
	2004	ns	ns	0.011	0.029	0.012	ns

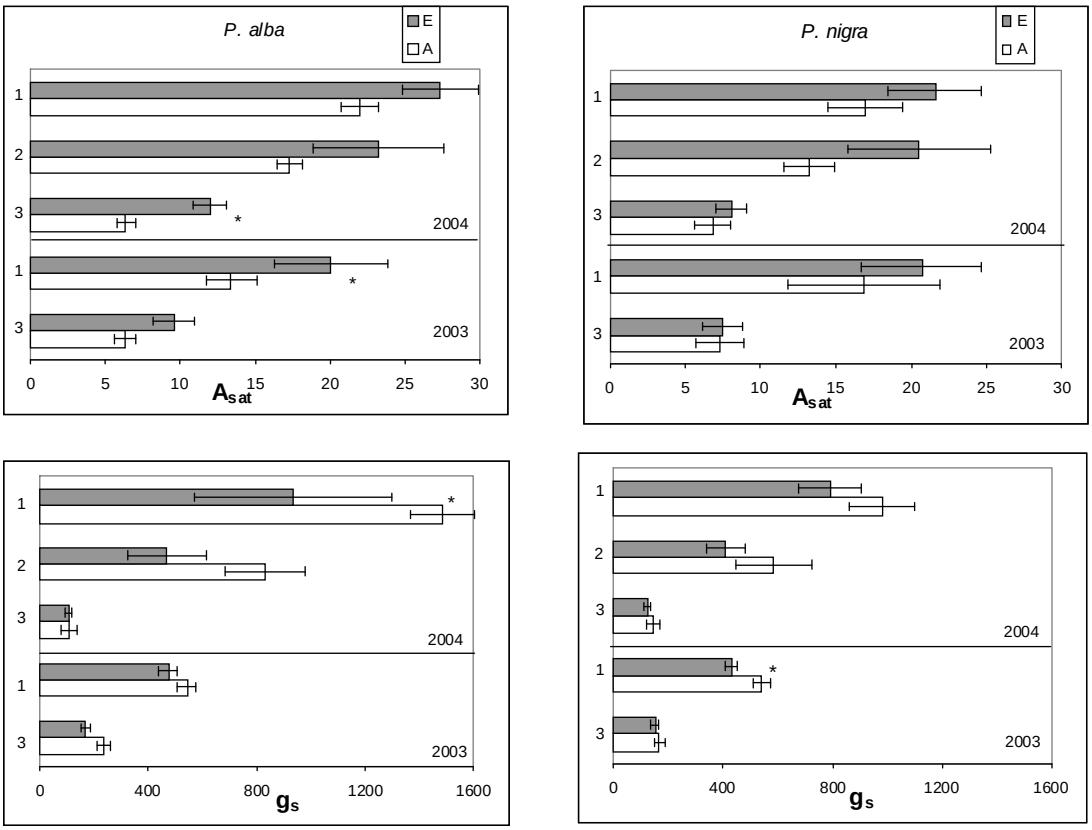

Figure 2. Light-saturated net CO_2 assimilation at growth CO_2 concentration, Asat, (μmol m^{-2} s^{-1}) and in situ stomatal conductance (g_s, μmol m^{-2} s^{-1}) in leaves from different treatments and canopy levels. Otherwise as in Figure 1

did not qualitatively affect the results (data not shown).

Stomatal conductance (g_s) was significantly reduced by CO_2 enrichment (Table 2, Fig. 2). In *P. alba*, elevated CO_2 resulted in reductions of in situ abaxial gs in sun and shade leaves of 13% and 28%, respectively. The corresponding reductions for *P. nigra* were 20% and 9%.

Leaf N partitioning within the photosynthetic system was influenced by canopy layer (Table 2, Fig. 3): shade leaves had higher P_L, but lower P_R and P_B than corresponding sun leaves. Partitioning between photosynthetic and non-photosynthetic N pools was not significantly influenced by either canopy layer or CO_2 enrichment.

September 2004

A significant positive effect of CO_2 enrichment on A_{sat} was detected in *P. alba* (Table 2, Fig. 2), while the stimulative effect in *P. nigra* was insignificant as was in 2003. The effects of CO_2 enrichment on the values of A_{400}, V_{cmax} and J_{max} (expressed either per unit leaf area or per unit mass) were insignificant (Table 2). Stomatal conductance was significantly reduced by CO_2 enrichment in *P. alba* (4-44% in the different canopy layers), whereas this reduction (14-30%) was insignificant in *P. nigra* (Table 2, Fig. 2). Stomatal density did not change in response to CO_2 enrichment in either of the studied species (Table 2). The significant interaction between CO_2 treatment and canopy layer for stomatal pl in *P. alba* resulted from a considerable reduction in pl in response to elevated CO_2 in the upper and middle canopy layers, but a small increase in the lower canopy layer. When all canopy layers were pooled, stomatal pl decreased considerably in elevated CO_2 conditions in *P. alba*, but not in *P. nigra*.

Elevated CO_2 significantly affected the pattern of N partitioning in *P. nigra*: compared with leaves grown in ambient CO_2 conditions, less leaf N was partitioned into Rubisco and more into non-photosynthetic N (Table 2, Fig. 3). The effect of canopy layer was also strong in 2004, with higher values of LMA, N_A, N_M, A_{sat}, A_{400}, V_{cmax}, J_{max}, g_s and D_s, but lower values of stomatal pl, detected in the upper canopy layers (Table 2).

When data from the two years were combined, there was a strong (42%) significant effect of elevat-

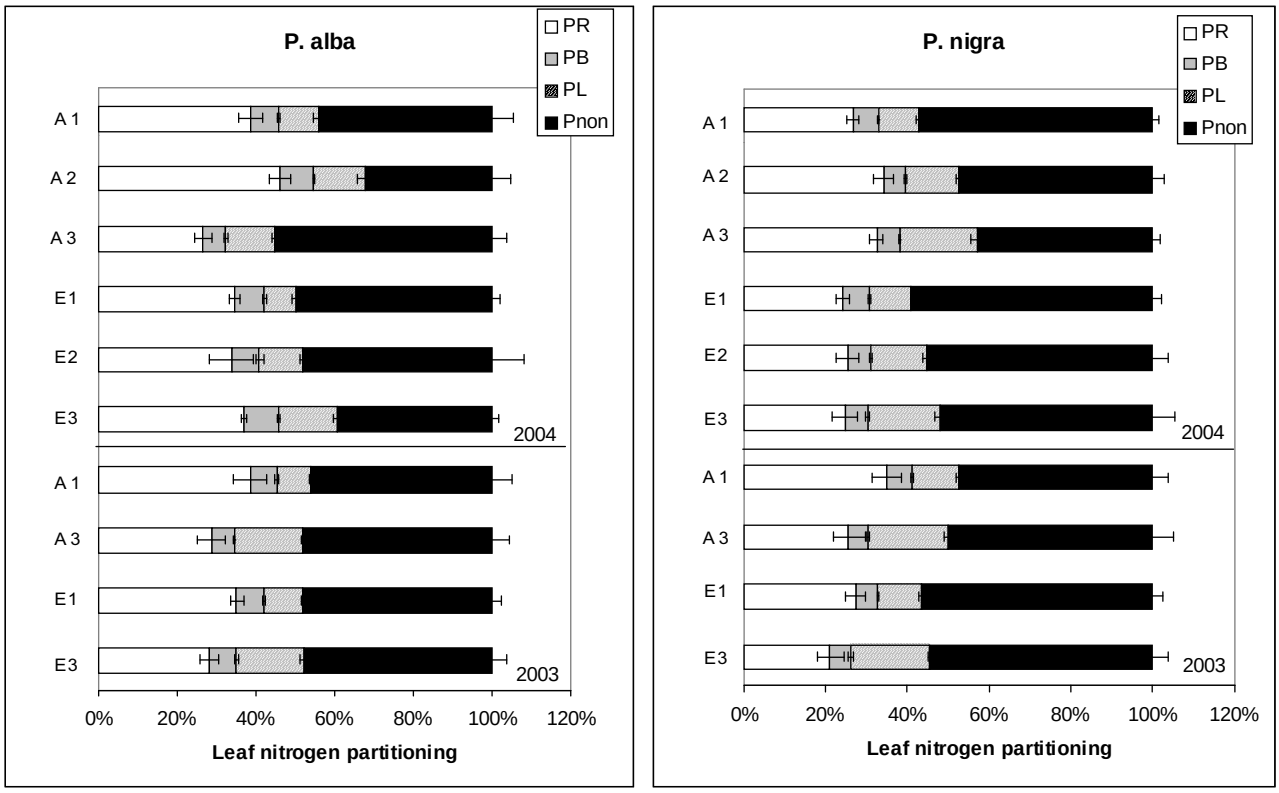

Figure 3. Leaf nitrogen partitioning. The vertical axis presents combinations of CO_2 treatment (E=elevated CO_2; A=ambient CO_2) and canopy layer (1=uppermost; 2=middle (in 2004 only); 3=lowest). P_R=partitioning of leaf nitrogen into carboxylation, P_B=partitioning into bioenergetics associated with electron transport, P_L=partitioning into thylakoid light-harvesting components and P_{non}=partitioning into non-photosynthetic pool. The standard error of P_{non} is the propagated error of P_R, P_B and P_L

ed CO_2 on A_{sat} in *P. alba* (Table 3). In *P. nigra*, photosynthetic stimulation in response to CO_2 enrichment was on average 29%, but this was not significant. Stomatal conductance was considerably reduced by elevated CO_2 in both species. The partitioning between photosynthetic and non-photosynthetic N pools was not affected by CO_2 enrichment in *P. alba*; by contrast, in *P. nigra*, the negative effect of elevated CO_2 on P_R and its positive effect on P_{non} were significant. The plants grown in elevated CO_2 tended to have lower photosynthetic rate when measured at ambient CO_2 (A_{400}) compared with plants grown and measured in ambient CO_2, but this difference was not significant, as also lower V_{cmax} and higher J_{max} under elevated CO_2 (Table 3).

Table 3. The effect of elevated CO_2 on studied leaf characteristics, expressed as the ratios of values in elevated CO_2 to those in ambient CO_2. Data are combined across years and canopy layers. Significance is indicated as * P<0.05

	P. alba	*P. nigra*
LMA	1.08	1.09
N_M	0.92	1.00
N_A	1.01	1.11
A_{sat}	1.42*	1.29
v_{cmax}	0.95	0.92
J_{max}	1.06	1.09
A_{400}	0.89	0.84
ISF	0.89	0.90
g_s	0.78*	0.82*
P_R	0.96	0.82*
P_B	1.10	1.00
P_L	1.02	0.91
P_{non}	1.01	1.15*

Discussion and conclusions

FACE reviews have reported average stimulation of leaf carbon assimilation due to elevated CO_2 concentrations in the range 26-31% (Nowak et al. 2004, Ainsworth and Rogers 2007), with woody species more responsive than herbaceous ones. Trees have also been found to show the least changes in V_{cmax} and J_{max} under the FACE regime (Ainsworth and Rogers 2007). In EUROFACE, significant photosynthetic stimulation has generally been found in elevated CO_2 treatments (Liberloo et al. 2007, Liberloo et al. 2009). Overall, we detected a significant positive effect (42%) of elevated CO_2 on A_{sat} in *P. alba*, but insignificant photosynthetic stimulation (29%) in *P. nigra*. Thus, during the second rotation, the photosynthetic stimulation of

poplars under CO_2 enrichment varied from no stimulation (all three species; Bernacchi et al. 2003) to 54% in *P. x euramericana* (Liberloo et al. 2007). The stimulation of total biomass production under elevated CO_2 conditions during the second rotation was, however, highest in *P. nigra* (Liberloo et al. 2006). Elevated CO_2 increased the leaf area index (LAI) of all species in 2003, with *P. nigra* most responsive (Liberloo et al. 2005). The high biomass production of *P. nigra* in elevated CO_2 is probably associated with high LAI and possibly also with significantly increased leaf photosynthesis during at least some periods of the second rotation.

Meta-analyses have identified photosynthetic down-regulation as a recurring phenomenon in elevated CO_2 experiments (Medlyn et al. 1999, Ainsworth and Long 2005, Ainsworth and Rogers 2007). However, recent EUROFACE articles have claimed that photosynthetic down-regulation does not occur in poplars under EUROFACE, because their sink capacity is large and N is not limiting (Liberloo et al. 2006, Liberloo et al. 2007). Davey et al. (2006) concluded that any down-regulation of photosynthetic capacity in poplars is short-term and transient, and is more likely to be associated with decreased activity than with decreased levels of photosynthetic enzymes. In accordance with this view, our results revealed no significant evidence of photosynthetic down-regulation in *P. alba* or *P. nigra* during 2003-2004. Nevertheless, we cannot be entirely certain that photosynthetic down-regulation did not occur, since several insignificant trends indicated that some responses may have remained undetected because of relatively small sample sizes. For example, plants grown in elevated CO_2 had lower V_{cmax} and lower photosynthetic rate when measured at ambient CO_2 compared with plants grown and measured in ambient CO_2 concentration.

Stomatal conductance was significantly reduced by CO_2 enrichment in both species. This appears to be a fairly general response to elevated CO_2 (Medlyn et al. 2001) and has also been found in EUROFACE experiments (Calfapietra et al. 2005, Tricker et al. 2005; though with some exceptions, Bernacchi et al. 2003). Reduction in stomatal conductance in response to CO_2 enrichment is generally believed to occur because of a reduction in stomatal apertures (controlled by the guard cells) and is therefore likely to be transient. However, Tricker et al. (2005) found that both the stomatal index and stomatal density were reduced by elevated CO_2 in the first 2 years of the first rotation. Our results from 2004 revealed no significant changes in stomatal density, but a tendency for stomatal pl in *P. alba* to decrease in elevated CO_2 conditions. If stomatal pl decreases, the reduction in stomatal conductance due to elevated CO_2 may be, at least partly, a long-term and persistent response.

N re-allocation between photosynthetic and non-photosynthetic leaf N pools was detected in P. nigra: more N was partitioned into the non-photosynthetic fraction as a result of growth in elevated CO_2. This re-allocation was significant in 2004 and in the combined data set, while a similar non-significant trend was observed in 2003. Greater allocation of leaf N into the non-photosynthetic pool is associated with downward shifts in the relationships between photosynthetic capacity (V_{cmax} and J_{max}) and leaf N (Medlyn et al. 1999). Such downward shifts were not evident in either 2003 (data not shown) or 2004 (Liberloo et al. 2007). An increase in non-photosynthetic N occurs during leaf senescence, when N is cycled from leaves to perennial plant tissues in the form of N-rich amino acids and other mobile nutrients (Cooke and Weih 2005). However, we found no evidence (lower chlorophyll concentrations, V_{cmax}, J_{max}) to suggest that leaves grown in elevated CO_2 conditions were physiologically older compared with those grown in ambient CO_2 conditions. In fact, delayed autumnal senescence has been found in EUROFACE in elevated CO_2 conditions (Taylor et al. 2008). Thus, neither the higher proportion of non-photosynthetic N in the leaves of *P. nigra* nor the insignificant photosynthetic stimulation in P. nigra that we observed in elevated CO_2 conditions can be explained by accelerated leaf senescence.

Light conditions strongly affected several leaf parameters (LMA, NM, NA, Asat, A400, V_{cmax}, J_{max}, g_s, D_s and stomatal pl), as is typical of shade-intolerant species (Kubiske et al. 2002). In addition, P_L was consistently higher in shade leaves, indicating that in low-light conditions more N was partitioned into chlorophyll. Differences in stomatal and photosynthetic responses to high CO_2 concentration between leaves from various canopy positions have been found in some previous studies (Kubiske et al. 1997, Kubiske et al. 2002), but not in others (Herrick and Thomas 2001, Herrick et al. 2004). In our study, differences in responses to CO_2 between the leaves from different canopy layers (Table 2, interaction CO_2×canopy layer) were associated with leaves from different layers responding to elevated CO_2 either in a similar direction but with a different magnitude, or in opposite directions. These few significant interactions between CO_2 treatment and canopy layer did not lead to differences in photosynthetic CO_2 responses between sun and shade leaves.

To conclude, the response of *P. alba* to elevated CO_2 was typical of trees with indeterminate growth in fertile soil conditions. Photosynthetic stimulation caused by growth in elevated CO_2 was considerable and

was sustained over two seasons with no significant biochemical down-regulation. Stomatal conductance in *P. alba* was significantly reduced in the elevated CO_2 treatment. In *P. nigra*, photosynthetic stimulation was non-significant. Greater allocation of leaf N into the non-photosynthetic fraction may be one reason for the non-significant photosynthetic stimulation in elevated CO_2 conditions exhibited by *P. nigra.*

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РЕАКЦИЯ ФОТОСИНТЕЗА НА ПОВЫШЕНИЕ КОНЦЕНТРАЦИИ CO_2 ТОПОЛЯ (ЭКСПЕРИМЕНТ „POP-EUROFACE") В ЗАВИСИМОСТИ ОТ РАСПРЕДЕЛЕНИЯ АЗОТА В ЛИСТЕ

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Резюме

В эксперименте POP-EUROFACE мы изучали эффекты влияния повышенной концентрации CO_2 на интенсивность фотосинтеза и распределения запасов азота, связанных и не связанных с фотосинтезом в листьях двух видов тополя (*Populus alba*, генотип 2AS11 и *Populus nigra*, генотип Jean Pourtet). У *P. alba* при полном освещении скорость фотосинтеза при увеличении концентрации CO_2 A_{sat}, в течении двух лет была значительно (42%) выше. У *P. nigra* при повышенной концентрации CO_2 не было такого резкого увеличения A_{sa} и в среднем A_{sat} увеличилось на 29%. Проводимость устьиц при повышенной концентрации CO_2 была значительно меньше у обоих видов: 22% у *P. alba* и 18% у *P. nigra*.

Повышенная концентрация CO_2 не снижала максимальной скорости карбоксилирования (V_{cmax}) и максимальной скорости электронного транспорта (J_{max}). У *P. nigra* было обнаружено изменение относительного запаса (количества) азота связанного с фотосинтетическими и нефотосинтетическими процессами: при повышенной концентрации CO_2 перераспределение на доли нефотосинтетического азота увеличивалась на 15%. Не обнаружена разница реакции освещённых и теневых листьев по фотосинтетическим параметрам. Повышенная аллокация азота в нефотосинтезирующих структурах листа при повышенных концентрациях CO_2 вероятно обьясняет незначительную стимуляцию фотосинтеза при повышении концентрации CO_2 у *P. nigra*.

Ключевые слова: скорость фотосинтеза, распределение азота, азота в нефотосинтезирующих структурах листа, *Populus*, адаптация фотосинтетического аппарата, проводимость устьиц